

PMC9 / Tag, T: D.V, DV.

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purpose: Amplification of PMC9 with different amounts of Tag titration.
 Deep vent
 Tag + Deep vent
 Constant
 different con - titration
 all samples discarded

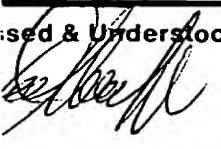
using dV primers, 1 μ M
 200 μ M dV
 1 μ M primer dV \times 2728 + 2729 (100 μ M)
 100 μ g template diluted 100 μ g / \times \rightarrow 1 μ g / \times
 Deep vent buffer

Titration: a. Tag: 0, 0.5, 1, 1.5, 2, 2.5 and 5 U
 b. Tag: Deep vent: 1:0, T1: 0.001, 1:0.05, 1:1.01, 1:1.05
 1:0.1, 1:2, 1:0.5, 1:1, 1:2
 c. Deep vent 0, 0.025, 0.05, 0.1, 0.5, 1, 2, 5

prepared a master mix w/o enzyme. Enzyme added separately.

Did just one of each.
 45 μ L + 5 μ L (enzyme + H₂O)
 H₂O 1167
 10 x buffer 150
 primer 1 15
 2 15

Tag: 5 U / \times diluted to 1 U / \times in 1x buffer. T. template 3 μ L
 Enzyme H₂O
 0 = - 5 D.V 2 U / \times \rightarrow 1 U / \times \rightarrow 0.1 U / \times 45 μ L
 1.5 = 0.5 4.5 0.001 U / \times
 1 = 1 4 0.025 2.5 } 0.01
 1.5 = 1.5 3.5 0.05 5 }
 2 = 2 3 0.1 1 } 0.1
 2.5 = 2.5 2.5 0.5 5 }
 5 = 5.0 - 1.0 0.5 } 2 U / \times
 2.0 = 4.0 5.0 = 2.5 μ L To Pag No. _____

Read & Understood by me,  Date 11/18/94
 Invented by _____ Date 11/18/94
 Recorded by K. Sitarman

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T	D.V	Tag	W/x	D.V	added in	Tube #
Unit 1	0	1	μl		1x super	16
	0.001			1	0.001 W/x	17
	0.005			5		18
	0.01			1	0.01 W/x	19
	0.05			5		20
	0.1			1	0.1 W/x	21
	0.5			5		22
	1.0			15	2.0 W/x	23
	2.0			1		24

- Thermocycled at 94°, 5'

30 (94°, 30", 56', 45 sec, 72', 3')

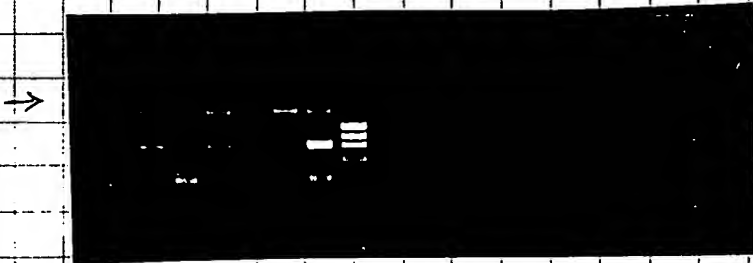
- after 6 cycles noticed dNTP was not added (1) & so added 1 μl of 10 mM dNTP (200 μM / 50 μM / 2X) individually each tube & started again the cycling!

- maybe have to repeat again.

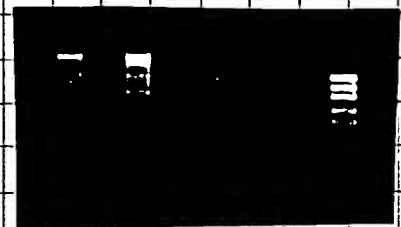
Tag alone

Deep vent alone

Tag: Deep vent



No - Contamination? X
(no enzyme)



0.001 0.005 0.01 0.05 0.1 0.5 1 2

- so much mispriming.

Repeat & in duplicate, use Lambda / Hind III marker

Witnessed & Understood by me,

Dat

11/28/94

Inv. nted by

R. corded by

R. Silberman

Dat

11/21/94

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